

Breeding for Resistance to Leafminer in Lettuce

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Abstract

Leafminer (*Liriomyza langei* Frick) is a major pest that causes considerable damage to a wide variety of vegetable crops including lettuce, and resistant cultivars remain the most economic means of insect control. Eighty-four lettuce cultivars and introduction lines were grown in an insect cage with eight replications for resistance screening. Leafminer flies were released in the cage to feed on the plants. Significant genetic variation for leafminer stings per unit leaf area was observed among genotypes tested. Resistant lines with fewer leafminer stings were found in *Lactuca sativa*, *L. saligna*, *L. serriola*, and *L. virosa*, and the resistance was confirmed in a field experiment. Crosses were made to combine leafminer resistance and superior horticultural traits in crisphead, green leaf, red leaf, romaine, and butterhead lettuces. Leaf miner resistant plants were selected in F₂ progenies of such crosses, and were backcrossed to restore horticultural type.

INTRODUCTION

Leafminers are major insect pests of many important agricultural crops (Parrella, 1987). They attack a wide range of plants including aster, baby's breath, bean, broccoli, cabbage, cauliflower, celery, chrysanthemum, cucumber, lettuce, muskmelon, onion, pea, potato, spinach, summer squash, Swiss chard, tomato, watermelon, and many weeds. The principal leafminer species affecting vegetables include *Liriomyza brassicae*, *L. sativae*, *L. trifolii*, *L. huidobrensis*, and *L. langei*. In the major lettuce production areas in central California, the predominant species was believed to be *L. huidobrensis* (pea leafminer) until recently (Morgan et al., 2000). Scheffer and co-workers (Scheffer et al., 2001) identified the leafminers in central California to be the morphologically cryptic species *L. langei* by using polymerase chain reaction (PCR) amplification of mitochondrial DNA.

Leafminer adults are small, shiny, black flies with a bright yellow triangular spot on the upper thorax between the wings. Damage occurs when adult flies puncture leaves to feed on plant sap and females lay white, oval eggs within the leaf tissue. Adult "stings" appear as holes or bumps on the leaves. Adult feeding on cotyledons may stunt seedling growth. Larvae hatch from eggs and feed between upper and lower leaf surfaces. The winding, whitish tunnels or mines they create are initially narrow, but they increase in width as the larvae grow. Larvae emerge from the mines after completing three instars and pupate in cracks in the soil or on the leaf surface. Adult flies come out of pupae in about 8 to 11 days. The entire life cycle can be completed in less than three weeks in warm weather. Many generations are produced each year in California. Adult sting and larval mining of leaves reduce photosynthetic capacity, render leaves unmarketable, and provide an entrance for disease organisms (University of California, 1992; LeStrange et al., 1999).

Few studies on leafminer resistance in vegetables have been reported in the literature. Larval (*L. trifolii*) antibiosis was found in four interspecific hybrids of *Lycopersicon pennellii*, *L. cheesmanii*, and *L. hirsutum*, and adult antibiosis and antixenosis for feeding was partially a result of the tomato plant's trichome exudates (Erb et al., 1993). No resistance to leafminers (*L. trifolii*) has been observed in cultivated celery, but

an accession from a wild species, *Apium prostratum*, was found to be practically immune (Trumble and Quiros, 1988). No feeding or oviposition was observed in this species. It has been used in a backcross program in an attempt to develop leafminer-resistant celery lines (Quiros, 1993). In addition, an accession from another wild species, *A. nodiflorum*, demonstrated substantial insect toxicity; few mines were observed and no larvae survived to the pupal stage (Trumble et al., 1990). In lettuce, significant differences among four romaine cultivars were found in the number of pupae produced and in the total numbers of stipples when exposed to leafminers (*L. trifolii*), but these differences resulted from differences in adult survival. Female leafminers survived significantly longer and produced more pupae on the cultivar Tall Guzmanine than on three other cultivars (Nuessly and Nagata, 1994; Nagata et al., 1998).

The genetic variation of leafminer resistance in the lettuce germplasm including the wild species has not been fully explored. The purposes of the present study were to evaluate differences in leafminer resistance among lettuce genotypes and to incorporate the resistance into elite lettuce cultivars.

MATERIALS AND METHODS

The experiments were conducted at the Agricultural Research Station of the U.S. Department of Agriculture, Salinas, CA in 2001. Eighty-four genotypes from the lettuce germplasm collection maintained at the station were used in this study. They include crisphead, leaf, romaine, butterhead, stem, Latin, Batavia, and primitive forms of cultivated lettuce (*Lactuca sativa*), and the wild species *L. serriola*, *L. saligna*, and *L. virosa* from different geographic areas of the world.

Three weeks after planting, 8 plants of each genotype were transplanted individually into plastic pots (10 x 10 x 10-cm) with soil. Plants were placed in field cages (2 m high by 4 m wide by 4 m deep) made of polypropylene shade cloth and were arranged in a randomized complete block with a single plant as the experimental unit and 8 replications.

Lettuce leaves with leafminer mines were collected from newly harvested fields around Salinas and leafminer larvae were allowed to emerge from the leaves and pupate. Pupae were collected, weighed, and put in plastic containers to allow the adult flies to emerge. The weight of a random sample of 200 pupae was used to estimate the number of pupae collected. The sampled pupae were also reared to adults to determine percentage of viable pupae. The number of flies was estimated based on weight and emergence of sampled pupae. About 2,500 flies were released in the field cage. The leaf with the most leafminer stings on each plant was counted for the number of stings per 20-cm² leaf area with the aid of an optical glass binocular magnifier (OptiVisor, Donegan Optical Co., Lenexa, Kans., USA) 7 days after the introduction of leafminer flies in the cage.

The genotypes were also transplanted in a field on the station in summer. Each plot consisted of six plants, with 30 cm between plants and 35 cm between rows on double-row beds of 1-m center. The experimental design was a randomized complete block with 8 replications. The leaf with the most leafminer stings on each plant was counted for the number of stings per 20-cm² leaf area when plants reached maturity. Data were analyzed statistically by using the Analysis of Variance (ANOVA) functions of the Microsoft Excel (Office 2001, Microsoft Co., Redmond, Wash., USA). Means of different lettuce genotypes were compared with *t* tests (Petersen, 1985).

RESULTS AND DISCUSSION

Significant differences among genotypes were found for the number of stings per unit leaf area, and the 11 genotypes with fewest stings are shown in Fig. 1. Two *L. saligna* lines (PI 509525 and PI 490999) had least leafminer stings among genotypes tested. Among the cultivated lettuces (*L. sativa*), PI 187238, PI 491212, Merlot, Lolla Rossa, and Salad Bowl showed fewer leafminer stings per unit leaf area than other lines. Da Ye Wo Sun, a stem lettuce from China, registered the most sting damage among the genotypes tested. These results demonstrate the existence of high levels of leafminer resistance in

different lettuce types and species. However, all genotypes had at least a few stings, indicating that none of the 84 genotypes tested was immune to leafminer.

Although leafminer resistance was shown in both wild species and cultivated lettuce, the transfer of resistance from wild species often brings in horticulturally undesirable traits. It may be easier to use the cultivated lettuce as source of resistance in a breeding program. However, genes from wild species can be used to broaden the genetic base of resistance.

In these choice tests, fewer stings per unit leaf area suggest host nonpreference or antixenotic resistance. Resistance based on antixenosis would be desirable because even the photosynthetic losses caused by adult feeding and oviposition would be reduced (Trumble et al., 1985). It could prompt leafminer movements to weeds or crops that are tolerant to insect damages. For example, broccoli or cauliflower (both important crops in central California) is rarely damaged by leafminers after the 6-leaf stage, regardless of population numbers (University of California, 1992).

The results from the cage test were confirmed in field experiment (Fig. 2). The plants in the cage experiment and in the field trial were at different ages, and were also subjected to different environments and leafminer pressures. Nevertheless, results of the stings per unit leaf area from the two tests were consistent. These similarities in performance demonstrated that differences in resistance were stable and a cage test can be used to screen lettuce plants for leafminer resistance.

Chemical control of leafminers usually lasts only a short period of time (Chaney, 2001), and many studies have shown that leafminers can develop a high degree of resistance to a broad range of insecticides (Parrella and Trumble, 1989; Keil and Parrella, 1990; Chaney, 2001). Resistant varieties remain the most economical means of insect control. Their use cuts down the costs of chemicals, machinery, fuel, and labor associated with pesticide spray. It may also reduce pesticide contamination of soil and ground water, alleviating the pressure on the environment.

We have developed a breeding scheme to incorporate the leafminer resistance into elite lettuce cultivars (Fig. 3). Resistant sources are crossed to cultivars of superior horticultural traits, followed by pedigree selection. Since some resistant sources are from wild species, it may be necessary to backcross to the adapted cultivars a few times to restore the horticultural traits. The progenies of the crosses can also be used to study the inheritance of leafminer resistance. Crosses are also made among the resistant sources, and their progenies are selected to elevate the level of resistance. Following such a scheme, a breeding program for leafminer resistance is in progress (Table 1). We have crossed resistance sources to crisphead, leaf, romaine, and butterhead type of lettuces, and selections or backcrosses have been made in the progenies.

In summary, a wide range of genetic variability was found in different types and species of lettuce. Coupled with the stability of the trait, genetic improvement of lettuce for leafminer resistance seems feasible. We are currently incorporating the resistance sources found in this study into elite cultivars in a lettuce breeding program.

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Tables

Table 1. Progress in breeding for resistance to leafminer.

Resistance source	Recurrent Parent	Current generation
PI 169513	Salinas (crisphead)	F ₃ and BC ₁ F ₂
Bibb	Salinas 88 (crisphead)	
Lolla Rossa	Tiber (crisphead)	
Red Grenoble	Lobjoits (romaine)	
Tom Thumb	Prizehead (leaf)	
PI 187238	Glacier (crisphead)	F ₂ and BC ₁ F ₁
PI 490999	Salinas	
PI 491178	Salinas 88	
PI 491212	Salad Bowl (leaf)	
PI 509525	Waldmann's Green (leaf)	
	Lolla Rossa (leaf)	
	Prizehead	
	Lobjoits	
	Parris Island (romaine)	
	Valmaine (romaine)	
	Dark Green Boston (butterhead)	
	Margarita (butterhead)	

Figures

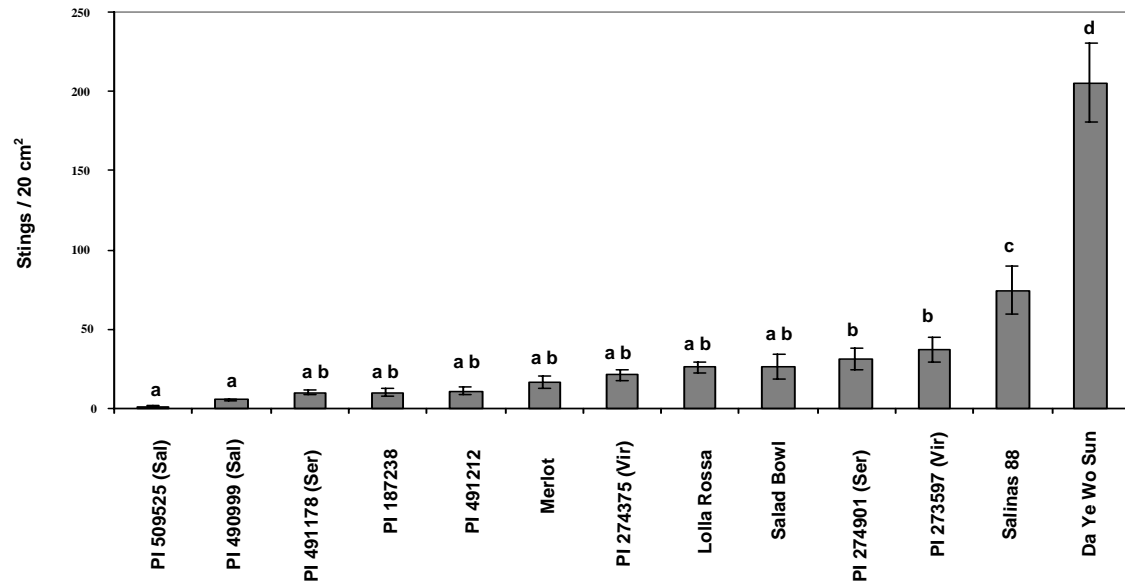


Fig. 1. Mean and standard errors of leafminer stings per 20 cm² leaf area of selected *L. sativa*, *L. saligna* (Sal), *L. serriola* (Ser), *L. virosa* (Vir) genotypes tested in an insect cage. Means with same letters are not significantly different at $P = 0.05$ level.

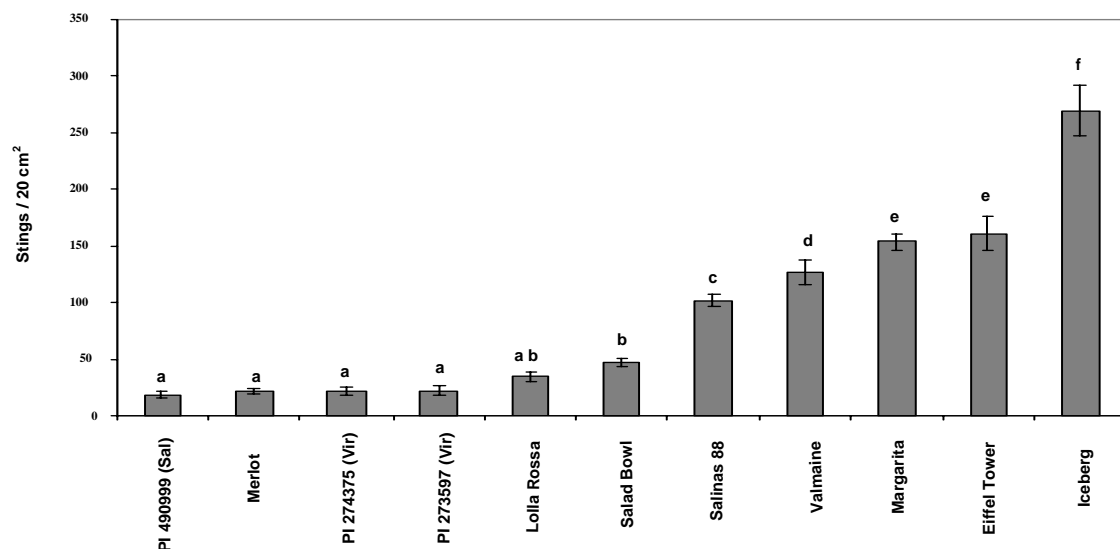


Fig. 2. Mean and standard errors of leafminer stings per 20 cm² leaf area of selected *L. sativa*, *L. saligna* (Sal), and *L. virosa* (Vir) genotypes tested in the field. Means with same letters are not significantly different at $P = 0.05$ level.

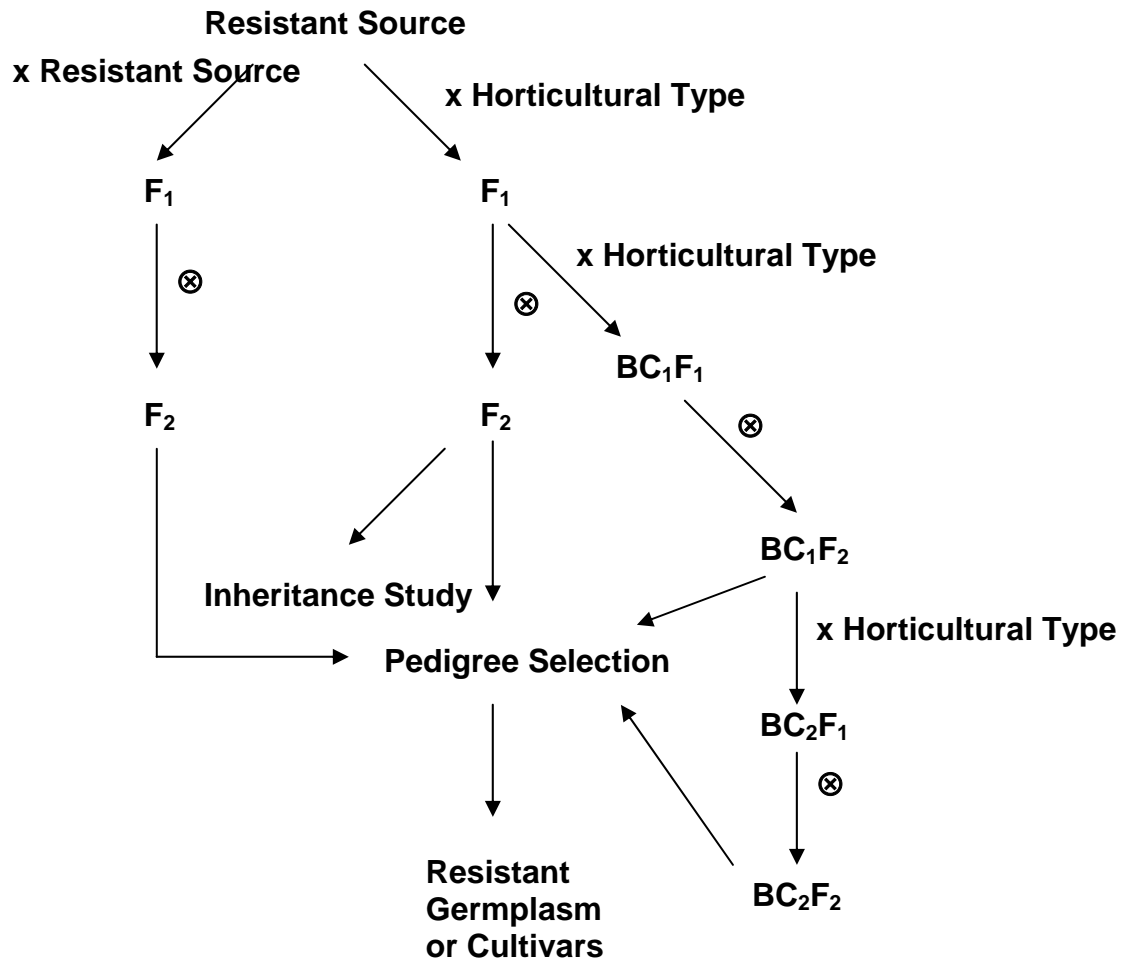


Fig. 3. Crossing and selection scheme for developing leafminer-resistant germplasms or cultivars, ⊗ = selfing, x = crossing.